(3) In the slow-channel syndrome, the degeneration of the junctional folds explains the loss of AChR and the reduced MEPP amplitude.[8,26]

Electron cytochemical localization of AChR with peroxidase-labeled α-bungarotoxin (α-bgt) or with anti-AChR antibodies displays the density and distribution of AChR over the postsynaptic membrane.[21,22,25,27,28] This information, together with the MEPP amplitude and the number of α-bgt binding sites per EP (see below), helps to assess the extent, significance, and mechanism of EP AChR deficiency.

^{125}I-α-bgt Binding Sites Per Endplate

This test is performed by incubating fine strips of muscle intact from origin to insertion in an oxygenated solution containing ^{125}I-α-bgt of known specific activity. After adequate rinsing, the strips are fixed and the EPs visualized by reacting them for AChE. After counting the EPs, the strips are divided into EP-positive and EP-negative segments of equal length and the radioactivity of the segments is measured in a gamma counter. The number of toxin molecules bound per endplate is then calculated from the difference in radioactivity between the EP-positive and EP-negative segments, the number of EPs, and the specific activity of the labeled toxin.

Two toxin molecules bind to each AChR. The number of toxin binding sites per EP, or N, is the product of the A_T x D, where A_T is the total postsynaptic membrane area that binds toxin, and D the average density (number per unit area) of binding sites. In infants and young children, N is smaller than in adults because the EPs, and therefore A_T, are smaller than in adults. In adults with endplates of normal shape and size, a decrease in N argues for a decrease in D. In diverse CMS, a decrease of N is associated with the appearance of multiple small EP regions over an extended span of the fiber surface.[19,21,22,26] Here the decrease must involve either A_T or D. (When N is reduced, D could be reduced and A_T reduced, normal, or increased; or D could remain constant while A_T is reduced.) Depending on the etiology, ultrastructural localization of peroxidase-labeled α-bgt at such EPs shows either a diffuse decrease of AChR over intact junctional folds[19,21,22] or a focal loss of AChR from degenerating folds.[26]

In Vitro Electrophysiology Studies

CONVENTIONAL MICROELECTRODE STUDIES

A muscle specimen intact from origin to insertion is obtained from an intercostal [29] or the anconeus muscle [30] and the amplitude, frequency, and decay time constants of the MEPP and MEPC as well as parameters of evoked quantal release are routinely determined.

Potentials are recorded from rested muscle with focally placed microelectrodes and their amplitude is normalized for a resting membrane potential of -80 mV. Potentials >3 mV are also corrected for nonlinear summation as described by Martin.[31] The MEPP amplitude is affected by the cable properties of the sarcolemma,[16] the number of ACh molecules per quantum, end-plate geometry, and the density and kinetic properties of AChR.[32,33] With AChE intact, the decay phase of the MEPP is related to the open time of the AChR channel[34,35] and the cable properties of the sarcolemma.[16,36] In children, the small diameter of the muscle fiber increases the input resistance of the fiber. In this case, the MEPP amplitude is corrected by the factor $(D_o/55)^{3/2}$, where D_o is the observed fiber diameter and 55 represents the normal mean adult fiber diameter in μm.[16]

The MEPC is recorded from the voltage-clamped muscle fiber. It is independent of the cable properties of the sarcolemma but otherwise is affected by the same factors as the MEPP.[37] The amplitude and duration of the MEPC are the macroscopic expression of the bursts of AChR channel activity occurring in response to one quantum of ACh.[38] With EP AChE intact, the decay time constant of the MEPC reflects, but is usually somewhat longer than, the mean burst duration.

The number of quanta released by a nerve impulse (m) is determined at 1 Hz stimulation.[31,39,40] When nerve stimulation causes the preparation to twitch, suitable amounts of curare are added to the bath. The 7th to 70th EPP is recorded from a train of 70 and m is calculated by the variance method, in which

$$m = (EPP_m)^2/EPP_V \quad (1)$$

where EPP_m and EPP_V are the mean and variance of the corrected EPP amplitudes,[40] or by the failures method, in which

$$m = \ln (\text{impulses/failures}) \quad (2)$$

When the amplitude of the EPP is subthreshold for eliciting a muscle fiber action potential, no curare is added to the bath and m can be obtained by the ratio method, in which

$$m = EPP_m/MEPP_m \quad (3)$$

When m exceeds 8 and is obtained from equations 1 or 2, it is also corrected for deviation from Poisson statistics by the empiric formula

$$m_c = 1.743 \times (m_o)^{0.733} \quad (4)$$

where m_c and m_o are the corrected and observed values of m.[41,42]

The value of m is affected by the probability of quantal release (p) and the number of readily releasable quanta (n), according to the formula $m = np$.[29,39] Therefore, p and n are also determined using brief trains of high-frequency stimuli.[43] The value of p is related to the calcium concentration within the nerve terminal. Under the experimental conditions, at a given EP n is affected by the total nerve terminal volume, the synaptic vesicle density, recruitment of synaptic vesicles to the readily releasable pool, priming of the vesicles for release, and the presynaptic membrane area available for vesicle exocytosis.

PATCH-CLAMP RECORDINGS

High resolution patch-clamp recordings of currents flowing through single AChR channels provide precise information on channel conductance and on the kinetic properties of the AChR.[19,26,44-49] Patch-clamp recordings, however, reflect the response of a limited number of channels to a fixed concentration of ACh at steady state whereas the MEPC reflects the instantaneous response to a pulse ACh at saturating concentration, and the opening probabilities of the AChR channel under the two conditions are different.[22]

Whole-cell patch-clamp recordings are useful in analysis of desensitization and ionic permeabilities of AChR. These recordings cannot be obtained at the EP but can be performed on fibroblasts transfected with wild-type or mutant AChRs.

Molecular Genetic Studies

Mutation analysis is greatly facilitated when the physiologic or morphologic studies point to a candidate protein whose primary sequence is known. For example, a kinetic abnormality of AChR detected at the single channel level,[26,28,45,46,50] or severe deficiency of AChR revealed by ^{125}I-α-bgt binding studies,[19,21,51] predicts one or two mutations in an AChR subunit gene. Absence of AChE from the EP predicts a mutation in the catalytic or collagenic tail subunit of the asymmetric form of AChE.[52] A predominantly limb-girdle distribution of weakness suggests a mutation in *DOK7*, *RAPSN*, or *GFPT1*. Sudden episodes of apnea point to a mutation in *CHAT*,[53] *RAPSN*,[23] *SCN4A*.[24] The pathogenicity of the mutations identified by the candidate protein approach is confirmed by cosegregation of the mutations with disease in the investigated kinship, absence of the identified variant from at least 200 alleles of 100 control subjects, and by expression studies in human embryonic kidney (HEK) cells,[54] *Xenopus* oocytes, monkey kidney fibroblasts (COS cells),[52] or mouse myotubes.[15]

When no candidate genes are apparent, mutation analysis can be based on frequencies of

the heretofore identified mutations in different endplate proteins, as shown in Table 1. This approach is more expensive and time intensive than the candidate gene approach. In our experience, about one-third of the DNA samples analyzed in this manner reveal no mutations.

Another approach is linkage analysis if a sufficient number of informative relatives are available. If successful, it will point to a candidate chromosomal locus. If the physical map of the locus shows an attractive candidate gene, then mutation analysis by direct sequencing becomes feasible. This approach seldom works for CMS because large informative CMS kinships are seldom available except for inbred populations with multiple consanguineous families,

A novel approach to mutation discovery is exome sequencing that searches for mutations in exons. Kits available for this method presently capture ~97% of the entire exome but read only 75% of the exome with more than 20x coverage and miss changes in noncoding DNA. The enormous amount of generated data need to be filtered against previously identified variants deemed nonpathogenic and selecting for mutations in genes that encode endplate related genes. The putative pathogenic mutations must still be confirmed by capillary sequencing and the pathogenicity of novel non-truncating mutations needs to be confirmed by expression studies. Moreover, exome sequencing is less efficient in detecting dominant than recessive mutations and is still very expensive. Sequencing the whole genome is also feasible but is even more expensive and more complicated to interpret than exome sequencing.[55]

A more direct and efficient approach is to use microarrays specifically designed for screening multiple candidate disease loci in known CMS genes. One publication finds this approach has a 73.3% overall sensitivity and a 95.5%a sensitivity for missense mutation, but it is not recommended for detecting insertion or deletion mutations.[56] Also, this approach will miss mutations in novel CMS disease genes.

EXPRESSION STUDIES

Once a mutation is identified and its pathogenicity confirmed, expression studies can provide information on how the mutation affects the level of expression, kinetic properties, and interaction of the mutant protein with other molecules. For example, coexpression of AChR subunit mutants with complementary wild-type subunits in HEK cells identifies null and low-expressor mutations, reveals whether the mutation interferes with subunit assembly, and shows how a mutation can affect the kinetic steps of receptor activation.[28,57] Coexpression of the collagenic tail mutants AChE with wild-type catalytic AChE subunits in COS cells demonstrates that the mutations prevent association of the tail subunit with the catalytic subunits, or prevents expression or assembly of the triple helical tail subunit required for insertion of the enzyme into the synaptic basal lamina.[52]

PRESYNAPTIC CMS

CMS Caused by Defects in Choline Acetyltransferase (ChAT)

CLINICAL FEATURES

The clinical features of this disorder were recognized more than five decades ago under the rubric of "familial infantile myasthenia",[58] but it was not differentiated from MG until the autoimmune origin of MG was established and electrophysiologic and morphologic differences were demonstrated between MG and the congenital syndrome.[5,6,59] Because the distinguishing clinical feature is *sudden and unexpected episodes* of severe dyspnea and bulbar weakness culminating in apnea, the disease has also been referred to as CMS with episodic apnea (CMS-EA). Initial studies of the clinical syndrome revealed no endplate AChR or AChE deficiency but suggested impaired resynthesis or vesicular packaging of ACh.[5,6]

Some patients present with hypotonia, bulbar paralysis and apnea at birth. Most patients gradually improve but still have variable ptosis, ophthalmoparesis, intermittent respiratory

difficulty and recurrent cyanotic episodes, some requiring resuscitation, during infancy and later life precipitated by infections, fever, excitement, or occurring with no apparent cause (Fig. 8-8-1). Few patients remain apneic and paralyzed since birth and some develop cerebral atrophy after episodes of hypoxemia.[60,61] Other patients are normal at birth and develop apneic attacks during infancy or childhood.[53,60-67] Some children after an acute attack experience respiratory insufficiency that may last for weeks.[68] Some patients are worsened by exposure to cold probably due to further decrease of the catalytic efficiency of the mutant enzyme at a lower temperature.[63] Between episodes of worsening, some patients appear normal or have only mild to moderate myasthenic symptoms. When weakness is absent, it can be readily induced by exercise. In the milder cases the crises become less frequent with age. After age 10, some patients only complain of easy fatigability on sustained exertion; others have mild to moderate weakness of cranial, limb, and respiratory muscles even at rest, resembling patients with mild to moderately severe autoimmune MG. The tendon reflexes remain normally active.[6,58,69-71] The disease is transmitted by autosomal recessive inheritance.[59]

Phenotypic heterogeneity can occur within a given kinship[62] or in unrelated patients carrying identical mutations.[65] Intrafamily phenotypic variability is illustrated by a kinship in which two sibs died suddenly at 2 and 11 months of age during febrile episodes; one was asymptomatic and the other had only mild ptosis prior to death. A third sibling began having abrupt episodes of dyspnea and cyanosis at age 14 months precipitated by fever or vaccination; at age 32 months, she developed ptosis and abnormal fatigue on exertion which lead to the diagnosis of a myasthenic disorder.[62]

ELECTROPHYSIOLOGY

A decremental response at 2 Hz stimulation and single fiber EMG (SFEMG) abnormalities are generally detected only when the tested muscles are weak. Weakness and EMG abnormalities consisting of a decrease of the CMAP to below 50% of the baseline and appearance of a decremental response at 2 Hz can be induced in some but not all muscles either by exercise or by subtetanic stimulation at 10 Hz for 5 to 10 minutes which is followed by *slow recovery* over 10 minutes or longer.[5,6,59,60] A marked decline of the CMAP during subtetanic stimulation also occurs in patients in other types of CMS but here the CMAP returns to the baseline within 1 to 2 min. Except in the most severely affected patients, the EMG decrement, *when present*, can be corrected by edrophonium.[70]

In vitro studies on intercostal muscle EPs elucidated the electrophysiologic basis of the disorder.[5,6,59] The MEPP amplitude is normal in the rested state, but it decreases abnormally after 10 Hz stimulation for 5 min. The amplitude of the EPP also decreases abnormally during 10-Hz stimulation and then recovers *slowly* over the next 10 to15 minutes (Fig. 8-2) whereas the quantal content of the EPP is essentially unaltered.[6,62]

MORPHOLOGY

Muscle biopsy specimens show no histochemical abnormality. The number of AChRs per endplate and postsynaptic ultrastructure are normal, but morphometric analysis indicates that the synaptic vesicles are smaller than normal in rested muscle.[6] The density and distribution of AChR on the junctional folds and the number of ^{125}I-α-bgt binding sites per EP are normal.[6,59]

MOLECULAR STUDIES

The slow recovery of the synaptic response to ACh after subtetanic stimulation pointed to a defect in the resynthesis or vesicular packaging of ACh and implicated four candidate genes: the presynaptic high-affinity choline transporter,[72,73] ChAT,[74] the vesicular ACh transporter

(VAChT)[75] and the vesicular proton pump.[76] In 2001, mutation analysis in 5 patients with characteristic clinical and EMG findings uncovered no mutations in *VACHT* but revealed 10 recessive mutations in *CHAT* that altered the expression or kinetic properties of the enzyme.[53] Subsequently similar clinical clues enabled different investigators to identify additional patients harboring *CHAT* mutations,[60,61,63-67] but none of these studies[60]examined the expression or kinetic properties of the mutant enzymes. In 2004, the atomic structural model of human ChAT was solved at 2.2 Å resolution[77,78] and kinetic effects of the mutations could now be related to their proximity to the substrate binding and catalytic sites of ChAT. Fig. 8-3A shows an atomic structural model of human ChAT and 12 missense and 1 nonsense mutation recently identified in our laboratory. Alone or in combination, the missense mutations alter the turnover rate, substrate affinity, substrate dissociation constant, or catalytic efficiency of ChAT, or render the enzyme conformationally unstable. Missense mutations positioned near the active site tunnel or the substrate binding sites of the enzyme have the most severe kinetic consequences[60] (See Fig. 8-3).

Patients harboring ChAT mutations have no autonomic symptoms or signs of central nervous system involvement other than attributable to anoxia. This cannot be due to ChAT having an EP-specific isoform because the observed mutations occur in the common coding region of all known ChAT isoforms. A possible explanation is that the ChAT level or substrate availability in the nerve terminal render ChAT rate limiting for ACh synthesis during physiologic activity at the EP but not at other cholinergic synapses. That stimulated quantal release at the EP is higher than at other cholinergic synapses likely contributes to selective vulnerability of the EP to reduced ACh resynthesis.

It is also important to note that defects in the presynaptic high-affinity choline transporter,[72,73] the vesicular ACh transporter,[75] or the vesicular proton pump,[76] could also curtail ACh resynthesis and result in similar clinical and EMG phenotypes, but no mutations of these proteins have been detected to date.

TREATMENT

Except in the few most severely affected patients, anticholinesterase medications benefit patients with myasthenic symptoms between respiratory crises, and prevent or mitigate the crises. Therefore prophylactic anticholinesterase therapy is advocated even for patients who are asymptomatic between crises. Some severely affected patients with permanent apnea and severe weakness that fail to respond to therapy harbor at least one mutation with severe kinetic consequences (Fig. 8-3B).

Parents of affected children must be indoctrinated to anticipate sudden worsening of the weakness and possible apnea with febrile illnesses, excitement, or overexertion. They also should be able to administer appropriate doses of prostigmine or pyridostigmine intramuscularly, and use an inflatable rescue bag with a fitted mask in a crisis and during transport to hospital. Long-term nocturnal apnea monitoring is indicated in any patient in whom ChAT deficiency is proven or suspected.[62]

Paucity of Synaptic Vesicles and Reduced Quantal Release

In this rare congenital myasthenic syndrome, the safety margin of neuromuscular transmission is compromised by the paucity of synaptic vesicles in the nerve terminal. The first instance of this disease was observed by us in 1989 in a 23-year-old woman with fatigable weakness of the bulbar and limb muscles since infancy[4] (Fig. 8-4). The symptoms responded to anticholinesterase drugs. Tests for anti-AChR antibodies were negative. A decremental EMG response was present at 2 Hz stimulation. In vitro microelectrode studies revealed that the quantal content of the EPP (m) was markedly reduced due to a decrease in the number of readily releasable quanta (n); the probability

of quantal release (*p*) was normal. The amplitude and the decay time constant of the MEPP were normal. Two observations indicated that the presynaptic voltage sensitive calcium channels functioned normally: (1) increased calcium concentration in the bath in which the excised muscle strips were incubated increased *m* normally and (2) increased potassium concentration in the bath increased the MEPP frequency normally. The number of AChRs per EP, estimated from the number of ^{125}I-α-bgt binding sites, was normal. Quantitative ultrastructural studies of unstimulated EPs demonstrated an approximately 80% decrease in synaptic vesicle density (no./μm^2) (Fig. 8-5), which was comparable to the decrease in *n*. Nerve terminal size, presynaptic membrane length, and the postsynaptic region were normal by ultrastructural criteria. A second patient with elements of the same disease was reported in an abstract in 1994 but the mutant protein was not identified.[79]

This syndrome superficially resembles the Lambert-Eaton myasthenic syndrome (LEMS) in that *m* is reduced in both, but unlike in LEMS (1) the amplitude of the initial compound muscle action potential (CMAP) is not reduced, (2) the CMAP does not facilitate appreciably at high rates of repetitive stimulation, (3) the decrease in *m* is due to a decrease in *n* (and not in *p*, as in LEMS), (4) the voltage sensitive calcium channels of the nerve terminal are functionally normal, and (5) the decrease in *n* is associated with a proportionate decrease in the density of the synaptic vesicles.

Synaptic vesicle precursors associated with different sets of synaptic vesicle proteins are produced in the perikaryon of the anterior horn cell and are carried distally along motor axons to the nerve terminal by kinesin-like motors.[80-83] Mature vesicles containing a full complement of vesicular proteins are assembled in the nerve terminal[83] and are then packed with ACh. After ACh has been released by exocytosis, the vesicle membranes are recycled and are repacked with ACh.[84] In the present syndrome the reduction in synaptic vesicle density could arise from (1) a defect in the formation of synaptic vesicle precursors in the anterior horn cell, (2) a defect in the axonal transport of one or more species of precursor vesicles, (3) impaired assembly of the mature synaptic vesicles from their precursors, or (4) impaired recycling of the synaptic vesicles in the nerve terminal. That synaptic vesicle density was reduced even in unstimulated nerve terminals argues against a defect in vesicle recycling.

Congenital Myasthenic Syndrome Resembling the Lambert-Eaton Syndrome

One young child was reported with this syndrome in 1987.[85] The CMAP amplitude was abnormally small but facilitated severalfold on tetanic stimulation, and the symptoms were improved by guanidine. A second patient observed at the Mayo Clinic was a 6-month-old girl with severe bulbar and limb weakness, hypotonia, areflexia, and respirator dependency since birth. The EMG showed a low-amplitude CMAP that facilitated 500% on high-frequency stimulation and decremented 40% on low-frequency stimulation. Studies of an anconeus muscle specimen revealed no EP AChR deficiency. Electron microscopy of the EPs showed structurally intact presynaptic and postsynaptic regions, no AChR deficiency, and abundant synaptic vesicles in the nerve terminals (Fig. 8-6). The MEPP amplitude was normal but the quantal content of the EPP, *m*, was less than 10% of normal at 1 Hz stimulation, and 40-Hz stimulation increased *m* by 300%. Thus, the in vitro electrophysiologic findings were like those in the Lambert-Eaton syndrome.[86]. Although 3,4-DAP which increases the number of ACh quanta released by nerve impulse[87] improved the EMG abnormalities, the patient remained weak and respirator dependent. The molecular basis of this CMS could be due to a defect in the presynaptic voltage-gated calcium channel $Ca_v2.1$ or in a component of the synaptic vesicle release complex. Mutation analysis of *CACNA1A* that encodes the pore forming α_1 subunit of the $Ca_v2.1$ revealed no mutations.

SYNAPTIC BASAL-LAMINA-ASSOCIATED CMS

Defects in three components of the synaptic basal lamina, AChE, β2 laminin and neural agrin,

are associated with CMS. This section considers the CMS caused by defects in AChE and β2-laminin. The CMS caused by mutations in agrin will be discussed in conjunction with defects postsynaptic proteins required for aggregation and anchoring of AChR in the postsynaptic region.

Endplate Acetylcholinesterase Deficiency

The EP species of AChE is an asymmetric enzyme composed of homotetramers of catalytic subunits ($AChE_T$) and a collagenic tail subunit composed of thee strands of ColQ. The tails subunit anchors the enzyme in the synaptic basal lamina.[88]

CLINICAL ASPECTS

Human EP AChE deficiency was first recognized in 1977 in a boy with life-long myasthenic symptoms refractory to AChE inhibitors.[7] AChE was absent from the endplates by enzyme cytochemical and immunocytochemical criteria, and electron cytochemical studies revealed no reaction product for the enzyme in the synaptic space. In most patients, weakness and abnormal fatigability are present since birth or early childhood and are highly disabling.[14,52,89,90] In the more severely affected patients poor suck, cry, and episodes of respiratory distress occur in infancy and motor milestones are delayed. In less severely affected patients the disease presents in childhood and becomes disabling only in the second decade [91-93] or later in life.[90] The weakness affects the facial, cervical, axial, and limb muscles (Fig. 8-7). Ophthalmoparesis is present but not in all patients. The axial muscles are severely involved, so that on standing the patient may show increasing lordosis and scoliosis after a few seconds. Fixed scoliosis and severe weakness and atrophy of the dorsal forearm and intrinsic hand muscles occur in older patients. In few patients, however, the weakness has a limb-girdle distribution. The tendon reflexes can be normal or depressed. Some patients have an abnormally slow pupillary light reflex. Phenotypic heterogeneity with regard to age of onset, progression, and severity of symptoms has been documented within and between kinships carrying the homozygous G240X mutation[90] or heterozygous[94] *COLQ* mutations.

ELECTROPHYSIOLOGY

The EMG shows a decremental response at 2 Hz (Fig. 8-8A) and at higher frequencies of stimulation in all muscles. Most patients have a repetitive CMAP response on nerve stimulation. The repetitive CMAP decrements faster than the primary CMAP and disappears at stimulation frequencies greater than 0.2 Hz (Fig. 8-8A) or with mild activity. Therefore it can be overlooked unless a well rested muscle is tested by single nerve stimuli.

In vitro microelectrode studies of intercostal muscles show the MEPP and MEPC amplitudes to be normal or moderately reduced. However, absence of AChE from the EP predicts a higher than normal MEPP and MEPCs amplitude. This discrepancy can be attributed to degeneration of the junctional folds with loss of AChR. The decay time constants of the MEPPs, EPPs, and MEPCs (Fig. 8-8B) are prolonged two- to three-fold compared normal subjects. Consistent with the absence of AChE from the EP, prostigmine has no effect on the amplitude or decay of the EP potentials or currents.[7,14] The quantal content of the EPP is markedly decreased due to a decreased number of releasable quanta (n); the probability of quantal release (p) is normal or higher than normal.[7,14,52,90,95] Patch-clamp analysis of single-channel currents indicates that the conductance and kinetic properties of the AChR channel are normal.[52,90]

MORPHOLOGY

Conventional histologic studies of muscle show type 2 fiber atrophy, or type 1 fiber preponderance, or both, or are normal. In most cases, AChE is absent from the endplate by light microscopic

criteria[7,14,90] but traces of AChE appear in some patients with C-terminal mutations in ColQ.[91] Electron cytochemical studies show no or only trace AChE in the synaptic cleft[7,14] (Fig. 8-9) but at some EPs sparse reaction product for AChE appears in the junctional sarcoplasm. Immunoreactivity for AChE with polyclonal and several monoclonal AChE antibodies is absent or barely detectable.[14]

Electron microscopy reveals that many nerve terminals are abnormally small. Also, at many EPs Schwann cell processes extend into the primary synaptic cleft and partially or even completely occlude the presynaptic membrane, reducing the surface available for ACh release (Fig. 8-10). At some EPs the junctional folds are honeycombed by myriad pinocytotic vesicles and labyrinthine membranous networks (Fig. 8-11A). At other EPs, the junctional folds are degenerating and shed AChR-rich fragments into the synaptic space with loss of AChR (Figs. 8-10 and 11B). Some of the junctional nuclei are degenerating or apoptotic. (Fig. 8-11A) The total number of AChRs per EP is normal or reduced.

PATHOPHYSIOLOGY

Because AChE is absent from the EP, AChR-ACh interactions are terminated by diffusion of ACh from the synaptic space. Before leaving the synaptic space, ACh binds to several AChRs and this prolongs the decay phase of the EP potentials and currents.[96] The prolonged EPP triggers one or more additional muscle fiber action potentials if its amplitude remains above threshold when the muscle fiber recovers from the refractory period of the preceding action potential.

The reduced number of readily releasable quanta (n) is adequately accounted for by the smallness of the nerve terminal (see Fig. 8-11 A and B) and the reduced presynaptic membrane surface available for ACh release (see Fig. 8-10). However, the smallness of the nerve terminals and the decrease in n are not as constant as the AChE deficiency.

AChR is lost from the EPs with degeneration of the junctional folds. The degenerative changes can be attributed to cationic overloading of the postsynaptic region by the increased synaptic activity,[97,98] but the EPs are partially protected from this by the restricted release of ACh quanta from the nerve terminal.

The safety margin is compromised by (1) smallness of n, (2) AChR deficiency, (3) desensitization of AChR from prolonged exposure to ACh during physiological activity,[99,100] and (4) progressive depolarization of the postsynaptic region during physiological activity. The depolarization may be similar to that observed in organophosphate poisoning,[101] arising from staircase summation of the prolonged EPPs at physiological rates of motor nerve firing. Progressive depolarization of the postsynaptic region inactivates the perijunctional voltage-sensitive sodium channels[102] which blocks the generation of the muscle fiber action potential.

MOLECULAR PATHOGENESIS

The endplate species of AChE is an asymmetric enzyme composed of homotetramers of globular (G) catalytic subunits attached to a collagenic tail subunit.[103] The catalytic subunit has two carboxyl-terminal splice variants, $AChE_T$ and $AChE_H$, expressed in muscle and erythrocytes respectively.[104] The collagenic tail subunit is formed by the triple helical association of three collagen-like strands, ColQ, encoded by *COLQ*, each of which can bind a homotetramer of $AChE_T$ to form the asymmetric A_4, A_8, and A_{12} moieties of the asymmetric enzyme[105] (Fig. 8-12A and B). Expression of globular and asymmetric forms of AChE in muscle, or in COS cells transfected with $ACHE_T$ and *COLQ* cDNA, is readily monitored by density gradient centrifugation of tissue or cell extracts (Fig. 8-12C and D)

Conserved domains of ColQ include an N-terminal proline-rich attachment domain (PRAD) that associates with an $AChE_T$ tetramer, a central collagen domain composed of GXY

triplets (where X and Y are any amino acids), and a C-terminal region enriched in charged residues and cysteines required for the assembly of the ColQ strands in a triple helix [106] (Fig. 8-12B). Anchorage of the asymmetric enzyme in the synaptic space is assured by two cationic heparan sulfate proteoglycan binding domains within the collagen domain[107] and by residues in the carboxyl-terminal domain.[89,95] The tail subunit is anchored to the synaptic basal lamina by at least two binding partners: the heparan sulfate proteoglycan perlecan,[108] which in turn binds dystroglycan, and the extracellular domain of MuSK.[109] Association with these binding partners predicts close proximity of the extracellular asymmetric enzyme to the postsynaptic membrane. All naturally occurring mutations in the EP species of AChE observed to date reside in ColQ.

Numerous *COLQ* mutations have been identified to date [52,90-92,94,95,110-112] (Fig. 8-12B). Four major types of mutations have been delineated by density gradient centrifugation analysis of extracts of COS cells cotransfected with cDNA of wild-type $ACHE_T$ and mutant or wild type *COLQ*. (1) Mutations involving PRAD prevent attachment of $AChE_T$ to ColQ and yield a sedimentation profile identical to that obtained after transfection with $ACHE_T$ alone (Fig. 8-12E and I) indicating that mutant ColQ, if expressed, fails to bind catalytic subunits[52,95] and no asymmetric AChE is formed. (2) Mutations that truncate the collagen domain prevent triple helical association of ColQ strands and give rise to an insertion incompetent truncated single strand of ColQ linked to an $AChE_T$ tetramer that sediments as a distinct mutant peak at 10.5S[52,95] (Fig. 8-12F and J). (3) A carboxyl-terminal mutant, 1082delC, produces a single-stranded insertion incompetent enzyme[52] on account of 64 hydrophobic missense residues that follow the frame-shifting point mutation [95] (Fig. 8-12G and K, left). (4) Other C-terminal mutations produce either reduced (R315X)[92] or normal amounts of the triple-helical asymmetric enzyme (Fig. 8-12H and K, right) which is generally insertion incompetent.[89]

DIAGNOSIS

A lifelong history of weakness and fatigability of all muscles, a decremental EMG response at all frequencies of stimulation, and refractoriness to anticholinesterase drugs should suggest the diagnosis of EP AChE deficiency. A repetitive CMAP response to single nerve stimuli in a patient not exposed to anticholinesterase drugs indicates EP AChE deficiency or a slow-channel syndrome but is not seen in all patients. The diagnosis is established by showing that AChE is absent from all EPs by cytochemical or immunocytochemical criteria. In vitro electrophysiological studies can further confirm the diagnosis by demonstrating typical abnormalities of the endplate potentials and currents. Alternatively, the diagnosis can be established by mutation analysis of *COLQ*.

THERAPY

There is no satisfactory therapy for EP AChE deficiency. Anticholinesterase medications have no effect on neuromuscular transmission and can cause excessive muscarinic side effects. If the diagnosis of AChE deficiency is *not* suspected, refractoriness to an anti-AChE medication may prompt the physician to increase the dose; this, in turn, may result in excessive bronchial secretions and worsen the patient's clinical state. Quinidine or fluoxetine which shorten the open duration of the AChR channel and benefit the slow-channel syndrome,[113,114] can cause increased weakness. Alternate-day prednisone therapy had a slight beneficial effect in two patients but was ineffective in one and appeared to worsen the symptoms in another. A respirator dependent infant with severe EP AChE deficiency was improved by intermittent blockade of AChR by atracurium, an agent that protects AChR from overexposure to ACh, allowing for temporary withdrawal of respiratory support.[115] Ephedrine sulfate at a dose of 150 to 200 mg/day in adults has a markedly beneficial effect in some patients.[93,112,116] Because ephedrine is no longer available in the US, the author has used oral albuterol sulfate, 8 to 16 mg per day in divided doses in adults with results

comparable to those of ephedrine. Vintage Pharmaceuticals suggests that dosing with albuterol sulfate in children 2 to 5 years of age should be initiated at 0.1 mg/kg of body weight three times a day. This starting dosage should not exceed 2 mg (1 teaspoonful of a syrup) three times a day.

Defect in β2-Laminin

Laminins are cruciform heterotrimeric glycoproteins composed of a central α and flanking β and γ strands and are assembled from products of five α, □four β, and three γ genes. The three identified laminins in synaptic basal lamina, laminin-4 (α2β2γ1), laminin-9 (α4β2γ1) and laminin-11 (α5β2γ1), contain β2 subunits associated with different α and γ subunits. Laminin 9 is restricted to the primary synaptic cleft and promotes the precise alignment of pre- and postsynaptic specializations. Laminin 11 lines the primary and secondary clefts, promotes presynaptic differentiation, and prevents Schwann cells from entering the synaptic cleft. The synaptic laminins provide a stop signal for axons at developing EPs and organize presynaptic differentiation.[117] Mice with targeted deletions of *LAMB2* that encodes β2-lamininin show simplified presynaptic nerve endings with a decreased number of active zones, no clustering of the synaptic vesicles above the active zones, and extension of Schwann cell processes into the primary synaptic cleft.[118,119] The MEPP frequency and quantal release by nerve impulse are reduced.[120]

In addition to its presence at the EP, β2-laminin is also highly expressed in renal glomeruli and the eye. *LAMB2* mutations in humans cause Pierson syndrome associated with ocular malformation (small nonreactive pupils, loss of accommodation, and abnormalities of the lens, cornea and retina) and a nephrotic syndromes fatal during infancy unless treated by a renal transplant (MIM 609049).

Recently Maselli and coworkers reported a 20-year-old woman with Pierson syndrome caused by two heteroallelic frameshifting mutations (1478delG and 4804delC) in *LAMB2* who also had a severe CMS.[121] The nephrotic syndrome was corrected by a renal transplant at age 15 months. The patient had respiratory distress in infancy, delayed motor milestones, a decremental EMG response, limited ocular ductions, bilateral ptosis, severe proximal limb weakness, scoliosis, and required assisted ventilation at night and sometimes during the day. Notably, her condition was worsened by pyridostigmine but was improved by ephedrine. Morphologic and microelectrode studies of the anconeus muscle revealed findings similar to those found in mice with targeted deletion of *LAMB2*.

POSTSYNAPTIC CMS

The presently identified postsynaptic CMS arise from defects in AChR, rapsyn, the muscle specific tyrosine kinase (MuSK), Dok-7, plectin, and $Na_v1.4$, the voltage-gated sodium channel of adult muscle. Rapsyn under the influence of agrin, LRP4, Dok-7, and MuSK maintains a high concentration of AChR in the postsynaptic membrane by linking the AChR to the subsynaptic cytoskeleton. MuSK as well as Dok-7 are important for maturation and maintenance of the neuromuscular junction. Plectin is an intermediate filament linker protein concentrated at sites of mechanical stress. At the EP, it provides crucial structural support for the junctional folds.

CMS Caused By Mutations in AChR

Most postsynaptic CMS are caused by one or more mutations in an AChR subunit gene that decrease the expression or alter the kinetic properties of the receptor. The kinetic mutations fall into two distinct groups: (1) dominant, gain-of-function mutations that prolong the openings of the AChR channel and cause slow-channel syndromes, and (2) recessive loss-of function

mutations that shorten the openings of the AChR channel and cause fast-channel syndromes. Some low expressor mutations also have minor kinetic effects, and some kinetic mutations also reduce AChR expression. This chapter discusses the CMS caused by low-expressor mutations and the phenotypic consequences of the kinetic mutations. Chapter 10 describes the structural features of the AChR and analyzes the mechanistic consequences of the kinetic mutations.

Slow-Channel Syndromes

CLINICAL ASPECTS

The slow-channel syndrome (SCCMS) was recognized by Engel and coworkers in 1982.[8] The distinguishing phenotypic features are dominant inheritance, selectively severe weakness of cervical, scapular, and finger extensor muscles, and variable weakness of other muscles. The affected muscles fatigue abnormally and are atrophic (Figs. 8-13). Except for the more severely affected patients, the cranial muscles are only mildly affected. The weakness and fatigability fluctuates but not as rapidly as in autoimmune MG. The tendon reflexes are usually normal but can be reduced in severely affected limbs. The severely affected muscles become atrophic. Progressive spinal deformities and respiratory embarrassment are common complications during the evolution of the illness. Some slow-channel CMS present in early life and cause severe disability by the end of the first decade;[26] others present later in life and progresses gradually or in an intermittent manner, remaining quiescent for years or decades between periods of worsening.[8,45,46]

ELECROPHYSIOLOGY FEATURES

As in endplate AChE deficiency, single nerve stimuli evoke a repetitive CMAP that decrements abnormally on repetitive nerve stimulation but there is no loss of EP AChE. The consecutive spikes of the repetitive CMAP occur at 5- to 8-ms intervals, each smaller than the preceding one, and disappear after a brief voluntary contraction (Fig. 8-14A). The repetitive CMAP was present in all muscles, except for one patient who is a somatic and germ-line mosaic for the εL269F mutation and has repetitive CMAPs only in proximal muscles. A decremental EMG response at 2 to 3 Hz stimulation is present in clinically affected muscles. The motor unit potentials fluctuate in shape and amplitude during voluntary activity.

In vitro microelectrode studies show the amplitude of the MEPP and MEPC significantly reduced in the more severely affected muscles. The quantal content of the EPP falls in the normal to low-normal range. Single-channel patch-clamp recordings demonstrate both normal and abnormally prolonged opening episodes of AChR. These reflect the activity of wild-type and mutant channels and account for the biexponential decay of the EP currents and potentials (Fig. 8-14B).[45,46,50] [26,49,122] Some mutant channels open even in the absence of ACh [26,50] (Fig. 8-14C), as predicted by the allosteric scheme of receptor activation, causing a continuous cation leak into the postsynaptic region.

MORPHOLOGY

Light microscopic histochemical studies in the SCCMS show type 1 fiber preponderance, isolated or small groups of atrophic fibers of either histochemical type, tubular aggregates, and vacuoles in fiber regions near EPs.[3,8] Other findings include abnormal variation in fiber size, fiber splitting, and sometimes mild to moderate increase of endomysial or perimysial connective tissue. AChE activity is present at all EPs. In the more severely affected muscles, the configuration of the EPs is often abnormal, with multiple small, discrete regions distributed over an extended length of the muscle fiber (Fig. 8-15A). In the most severe cases, focal calcium deposits can be demonstrated at the EPs with glyoxalbis-(*O*-hydroxyanyl) or alizarin-red dyes.[8]

On electron microscopy, the junctional folds of many EPs contain myriad pinocytotic

vesicles and labyrinthine membranous networks (Figs. 8-16A). At more severely affected EPs, the junctional folds are degenerating, causing a widening of the synaptic space, accumulation of electron-dense debris (Figs. 8-15B, 16B and D), and loss of AChR from the junctional folds (Fig. 8-16D). Some of the highly abnormal postsynaptic regions are denuded of their nerve terminals. Unmyelinated nerve sprouts appear near some EPs. The intramuscular nerves are normal. Degenerative changes also occur in the junctional sarcoplasm and in nearby fiber regions. These consist of the accumulation of membrane-bound vesicles (Fig. 8-16C), apoptotic nuclei (Fig. 8-16E), focal myofibrillar degeneration, and appearance of large membrane-bound vacuoles. Morphometric reconstruction of individual EP regions shows a significant decrease of nerve terminal size. The postsynaptic membrane length and density are reduced due to degeneration of the junctional folds.

PATHOPHYSIOLOGY

The prolonged EPPs, MEPPs, and MEPCs all stem from prolonged opening episodes of the AChR channel. As in congenital EP AChE deficiency, the repetitive CMAP can be explained by the prolonged EPP.

The prolonged opening episodes (Fig. 8-14B and 14C left) and spontaneous openings of the AChR channel (see Fig. 8-14C, right) result in abnormal ingress of cations into the junctional folds and nearby muscle fiber regions. For the normal adult human AChR, 7% of the synaptic current is carried by Ca^{2+}; this is higher than for human fetal AChR or for muscle AChR of other species, and predisposes to postsynaptic Ca^{2+} overloading when the synaptic current is prolonged. Slow-channel mutations in the α subunit do not augment the already high Ca^{2+} permeability of the receptor, but slow-channel mutations in the ε subunit do and thereby potentiate the deleterious effects of the prolonged synaptic currents and the intrinsically high Ca^{2+} permeability of the human receptor.[123,124] The focal Ca^{2+} excess exerts a deleterious effect on cellular proteins and membranes through activation of proteases such as the calpains, by promoting free radical production by activation of lipases or nitric oxide synthase,[125] and promotes apoptosis through activation of caspases and endonucleases.[126-128] This readily explains degeneration of the junctional folds, nuclear apoptosis, and other features of the endplate myopathy. The morphologic findings at slow-channel EPs resemble those at mouse muscle EPs exposed to carbachol, a cholinergic agonist, and the carbachol induced changes can be prevented by exclusion of calcium from the extracellular fluid.[129]

Multiple mechanisms compromise the safety margin of neuromuscular transmission: (1) Widening of the synaptic space causes diffusional loss of ACh and increases the chance of destruction of ACh by AChE. (2) Degeneration of the junctional folds results in loss of AChR. (3) Apoptosis of a proportion of junctional nuclei may compromise transcriptional regulation at the EP. (4) The marked tendency of some SCCMS mutants to desensitize (e.g., αV249F) predicts that an appreciable fraction of AChR is desensitized even in the resting state, further decreasing the number of receptors that can be activated. (5) The markedly prolonged decay of the EPPs (often longer than 40 ms) predicts their staircase summation and a depolarization block of transmission during even normal physiologic activity. (6) The spontaneous openings, or leakiness, of the mutant channels may partially depolarize the perijunctional Na^{+} channels, producing a depolarization block even at rest, and contribute to the cationic overloading of the postsynaptic region.

The structural and mechanistic features of the mutant slow-channel AChRs are detailed in Chapter 9.

MOLECULAR GENETIC SUDIES

The abnormal kinetic properties of AChR predicted that the slow-channel syndrome stemmed

from mutations in AChR subunits. Since 1995, numerous slow-channel mutations have been discovered.[26,45,46,49,122,130-138] The different mutations occur in different AChR subunits and in different functional domains of the subunits (Fig. 8-14D). Interestingly, a patient suffering from autoimmune myasthenia gravis had an acquired slow-channel syndrome attributed to an unusual kinetic effect of an anti-AChR antibody.[139]

Mutations in the channel domain have more severe phenotypic consequences than those at the ACh binding site. For example, a patient with the εT264P mutation in the second transmembrane domain (M2) of the receptor has been wheelchair dependent since her teens; a patient with the αN217K mutation in M1 is less severely affected but can only walk about 100 yards before having to rest; and patients with the αG153S mutation in the extracellular domain of AChR can still ski and play tennis in their 60s. However, there are also variations in phenotypic expressivity between and within kinships harboring the same mutation.[140] Thus, the mutation site is not a consistently reliable predictor of phenotypic severity.

DIAGNOSIS

The clinical diagnosis is supported by dominant inheritance, selective distribution of the weakness and fatigability, and a decremental and repetitive CMAP. A repetitive CMAP can also occur with EP AChE deficiency but here the repetitive response is typically single and unaffected by edrophonium whereas in the SCCMS it is often multiple and edrophonium increases the amplitude and number repetitive CMAPs. This and normal reactivity for AChE at the EP establishes the diagnosis of SCCMS. In vitro electrophysiological studies confirm the diagnosis by demonstrating abnormally slowly and biexponentially decaying MEPCs and abnormally prolonged opening events of single AChR channels. Misdiagnoses of SCCMS patients have included Möbius syndrome, peripheral neuropathy, radial nerve palsy, motor neuron disease, syringomyelia, mitochondrial myopathy, limb-girdle dystrophy, facioscapulohumeral dystrophy, and myotonic dystrophy. Careful assessment of the clinical and EMG features can exclude these entities.

THERAPY

Anticholinesterase drugs can provide temporary improvement but are ineffective or harmful in the long run. By further increasing the number of normal and abnormal receptors activated by ACh, AChE inhibitors enhance cationic overloading of the endplate and likely accelerate the progression of the endplate myopathy.

Long-lived open-channel blockers of AChR shorten the openings of the AChR channel and are thus ideally suited to treat the slow-channel syndrome. Quinidine proved to be such an agent [141] and attainable levels of the drug normalized prolonged opening episodes of slow-channel mutants expressed in human embryonic kidney (HEK) cells[113] (Fig. 8-17A and B). Based on this clue, Harper and Engel [114] treated slow-channel patients with 200 mg quinidine sulfate three to four times daily, producing serum levels of 0.7-2.5 μg/ml (2.1- 7.7 μM/L), and found that the patients improved gradually by clinical and EMG criteria. The discovery that fluoxetine blocks neuronal AChR channels,[142] prompted examination of its effects on opening episodes of slow-channel mutants expressed in HEK cells. This revealed that fluoxetine was another a long-lived open-channel blocker of muscle AChRs at clinically attainable levels (Fig. 8-17C and D) and pointed the way to successful therapy of SCCMS patients with 60 to 80 mg fluoxetine per day.[143] The safe use of both quinidine and fluoxetine requires monitoring the serum level and close observation of the patient for possible side effects. Fluoxetine has been reported to increase the risk of suicide-related behaviors in depressed children and adolescents.[144,145] Therefore caution is required when the medication is used in this age group, and it should not be used in patients with signs of depression. Because quinidine is now difficult to obtain commercially and

because it is prone to cause allergic reactions, the authors use fluoxetine to treat the SCCMS.

The structural and mechanistic features of the mutant slow-channel AChRs are detailed in Chapter 9.

Fast-Channel Syndromes

The fast-channel syndromes are caused by recessive loss-of function mutations that decrease affinity for ACh, or reduce gating efficiency, or destabilize channel kinetics, or act by a combination of these mechanisms. Each of these derangements results in abnormally brief channel opening events that are reflected by an abnormally fast decay of the synaptic response (Fig. 8-18A). A fast-channel mutation dominates the clinical phenotype when the second allele harbors a null mutation or if occurs at homozygosity. The fast channel mutations identified to date are shown in (Fig. 8-18B)

CLINICAL ASPECTS

The symptoms resemble those of autoimmune myasthenia gravis. They can be mild when the main effect is on gating efficiency,[48,146] moderately severe when channel kinetics are unstable,[22,147] and severe (Fig. 8-19) when affinity for ACh, or both affinity and gating efficiency, are impaired[28,47,148,149]

ELECTROPHYSIOLOGY FEATURES

The common electrophysiologic features of the fast-channel CMS are rapidly decaying low-amplitude endplate currents and abnormally brief channel activation episodes (Fig. 8-18A) The amplitude of the synaptic response is reduced by decreased agonist affinity, decreased gating efficiency, impaired gating fidelity, or a combination of these factors.[22,28,47,146,150]

The structural and mechanistic features of the mutant fast-channel AChRs are detailed in Chapter 9.

MORPHOLOGY

The low-affinity fast-channel syndromes caused by εP121L near the ACh binding site[28] and αV132L in the Cys-loop of the receptor[47] leave no anatomic footprint; the structural integrity of the EP is maintained, and there is no EP AChR deficiency (New Fig. 8-19). Those syndromes caused by the εN182Y or the εD175N mutation in the extracellular domain,[150] αV285I in the M3 domain,[146] and ε1254ins18 in the long cytoplasmic loop of the ε subunit,[22] are associated with variable decrease of AChR expression. These patients display multiple small EP regions dispersed over an extended length of the fiber surface, and some of the postsynaptic regions are simplified.

DIAGNOSIS

The specific diagnosis of a fast-channel syndrome requires in vitro microelectrode studies to show abnormally rapidly decaying MEPCs at voltage-clamped EPs, or the recording of abnormally brief channel openings from EP AChRs or from mutant AChRs engineered into HEK cells.

THERAPY

An attenuated postsynaptic response to ACh is common to all fast-channel mutations. Increasing the postsynaptic response is therefore the logical therapy. Indeed, most patients with fast-channel

CMS generally respond well to combined therapy with 3,4-diaminopyridine (3,4-DAP) which increases the number of quanta released by nerve impulse, and anticholinesterase drugs which increase the number of receptors activated by each quantum. Patients with a normal density of AChR on the junctional folds respond best, for a decreased density of receptors on the folds entails a proportionate reduction in the number of receptors that can be saturated by any given quantum. However, neither increasing the release of ACh quanta nor prolonging the lifetime of ACh in the synaptic space mitigates the deleterious effects of mutations at the ACh binding site This was observed in an 8-year-old girl with severe weakness of all voluntary muscles since birth and three similarly affected siblings who died in infancy. She carries a homozygous ε-subunit mutation that substitutes a positively charged arginine for an anionic tryptophan at codon 55 (εW55R). The mutated tryptophan is one of the aromatic residues that contributes pi-electrons to the anionic agonist binding site at the α/ε subunit interface. Compared to wild-type AChR, the mutation reduces agonist affinity 670-fold, decreases the channel opening probability to 1%, and shortens the channel burst open duration to 9%.

Combined therapy with pyridostigmine and 3,4-DAP was also of limited benefit in the case of a 4-year-old with life-threatening myasthenic symptoms since birth requiring frequent ventilatory support (Fig. 8-19). She carries an αV132L mutation in the highly conserved Cys-loop of the receptor[47] and a null mutation in the second allele of the α-subunit.

AChR Deficiency Caused by Recessive Mutations in AChR Subunits

CLINICAL FEATURES

The clinical phenotypes of patients with low expressor mutations in AChR subunit genes vary from mild to severe. Patients with recessive mutations in the ε subunit are generally less affected than those with mutations in other subunits, because expression of the fetal γ-subunit can compensate at least in part for the defect in the ε subunit. Low expressor or null mutations in both alleles of non-ε subunits cause very severe disease and often are lethal in embryonic or early life. The most severely affected patients have marked ocular, bulbar, and respiratory muscle weakness from birth and survive only with respiratory support and gavage feeding. They may be weaned from a respirator and begin to tolerate oral feedings during the first year of life, but they will have bouts of aspiration pneumonia and may need intermittent respiratory support during childhood and adult life. Motor milestones are severely delayed; they can seldom learn climb steps and can walk for only a short distance. Older patients close their mouth by supporting the jaw with their hand and elevate their eyelids with their fingers (Fig. 8-20). Facial deformities, prognathism, malocclusion, and scoliosis or kyphoscoliosis become noticeable during the second decade. Muscle bulk is reduced. The tendon reflexes are normal or hypoactive.

The least affected patients pass their motor milestones with slight or no delay and only show mild ptosis and limited ocular ductions. They are clumsy in sports, fatigue easily, and cannot run well, climb rope, or do pushups. In some instances, a myasthenic disorder is suspected only when the patient develops prolonged respiratory arrest on exposure to a curariform drug during a surgical procedure.

Patients with intermediate clinical phenotypes experience moderate physical handicaps from early childhood. Ocular palsies and ptosis of the lids become apparent during the first year of life. They fatigue easily and cannot keep up with their peers in sports, they walk and negotiate stairs with difficulty, but they can perform most activities of daily living (Fig. 8-21).

ENDPLATE STUDIES

Morphologic studies show an increased number of EP regions distributed over an increased span of the muscle fiber (Fig. 8-22A and B). The integrity of the junctional folds is preserved but

some EP regions are simplified and smaller than normal (Fig. 8-22C). The distribution of AChR on the junctional folds is patchy and the density of the reaction for AChR is attenuated compared to normal (Fig. 8-22C and D). Conventional microelectrode studies show a decreased amplitude of the miniature EP potentials and currents and frequently high or higher than normal quantal release by nerve impulse. Single channel recordings at the EP[19,151] or immunocytochemical studies[21] often reveal the presence of fetal γ-AChR (Fig 23).

MOLECULAR PATHOGENESIS

CMS with severe EP AChR deficiency result from different types homozygous or, more frequently, heterozygous recessive mutations in AChR subunit genes. The mutations are concentrated in the ε subunit (Fig. 8-24, lower panel). There are two likely reasons for this: (1) Expression of the fetal type γ subunit, although at a low level, partially compensates for absence of the ε subunit,[19,21,22] whereas patients harboring null mutations in non-ε subunits (Fig. 8-24, upper panel) often die early for lack of a substituting subunit. (2) The gene encoding the ε subunit, and especially exons coding for the long cytoplasmic loop, have a high GC content that predispose to DNA rearrangements.

GENETIC HETEROGENEITY

Different types of recessive mutations causing severe endplate AChR deficiency have been identified. Some mutations cause premature termination of the translational chain. These mutations are frameshifting,[19,21,47,51,152-155] occur at a splice site,[51,153] or produce a stop codon directly.[19] An important mutation in this group is the 1369delG in the ε subunit that results in loss of a C-terminal cysteine, C470, crucial to both maturation and surface expression of the adult receptor.[156] Thus any mutation that truncates the ε subunit upstream of C470 is predicted to inhibit ε expression.

Three recessive point mutations were identified in the Ets binding site, or N-box, of the promoter region of the ε subunit gene: ε-154G>A,[157], ε-155G>A,[158] and ε-156C>T.[159] The N-box represents the end point of a signaling cascade driven by neuregulin through ErbB receptors. ErbB receptors phosphorylate mitogen-activated protein (MAP) kinases. Phosphorylated MAP kinases phosphorylate GABPα and GABPβ (members of the Ets family of transcription factors), which then bind to the N box.[160-162] That these mutations impair AChR expression is direct evidence that the neuregulin signaling pathway participates in regulation of synapse-specific transcription at the human EP.

There are also missense mutations in a signal peptide region (εG-8R[28] and εV-13D[153]), and missense mutations involving residues essential for assembly of the pentameric receptor. Mutations of the latter type were observed in the ε subunit at an N-glycosylation site (εS143L)[28], in Cys 128 (εC128S) --a residue that is an essential part of the C128-C142 disulfide loop in the extracellular domain,[22] in Arg 147 (εR147L), which is part of a short extracellular span of residues that contributes to subunit assembly[19], in Thr 51 (εT51P)[153], and in the long cytoplasmic loop of the β subunit causing the deletion of three codons.[163] Another important missense mutation is δE381K in the long cytoplasmic loop of the δ subunit that causes clinical symptoms typical of rapsyn deficiency. Cotransfection of the δE381K-AChR with wild-type rapsyn showed reduced coclustering of the mutant receptor with rapsyn compared to wild type indicating the importance of δGlu381 as an AChR binding partner for rapsyn.[164]

Finally, it is important to note that some ε subunit mutations occurring at homozygosity are endemic in Mediterranean or other Near Eastern countries.[153,165] The frameshifting ε1267delG mutation occurring at homozygosity is endemic in Gypsy families[51,152,154] where it

derives from a common founder.[152]

THERAPY

Most patients respond favorably but incompletely to anticholinesterase medications. The additional use of 3,4-DAP (1 mg per kg per day given in divided doses every 3 to 5 hours) results in further improvement but the ocular ductions are often refractory to 3,4-DAP.[166] Perioral and distal paresthesias are common at the beginning of therapy. Convulsions are a rare but important complication of 3,4-DAP treatment; therefore, a potential or actual epileptiform abnormality on the electroencephalogram or a history of seizures contraindicate the use of the drug. 3,4-DAP can also prolong the QT interval; therefore its use is contraindicated in patients whose electrocardiogram shows a borderline prolonged or prolonged QT interval.

Escobar Syndrome

This is a prenatal myasthenic syndrome caused by recessive, nonsense, frameshift, splice site, or missense mutations in the fetal γ-subunit of AChR. In humans, γ-AChR appears on myotubes around the ninth developmental week and becomes concentrated at nascent nerve-muscle junctions around the sixteenth developmental week. Subsequently, the γ subunit is replaced by the adult ε subunit and is no longer present at fetal EPs after the thirty-first developmental week.[167] Thus pathogenic mutations of the γ-subunit result in hypomotility in utero mostly during the sixteenth and thirty-first developmental week. The clinical consequences at birth are multiple joint contractures, small muscle bulk, multiple pterygia (webbing of the neck, axilla, elbows, fingers, or popliteal fossa), fixed flexion contractures of the fingers (campodactyly), rocker-bottom feet with prominent heels, and characteristic faces with mild ptosis and a small mouth with downturned corners. Myasthenic symptoms are absent after birth because by then the normal adult ε subunit is expressed at the EPs.[167,168]

CMS Caused by Defects in Rapsyn

Rapsyn (receptor associated protein of the synapse), under the influence of agrin, LRP4, MuSK and Dok-7 concentrates AChR in the postsynaptic membrane and links it to the subsynaptic cytoskeleton through dystroglycan.[169-172] In myotubes, agrin, MuSK, and Dok-7, and possibly other myotube specific mechanisms, regulate rapsyn aggregation,[173] but rapsyn expressed in heterologous systems self-aggregates and can then recruit AChRs, dystroglycan, and MuSK.

The structural domains of rapsyn include an N-terminal a myristoylation signal required for membrane association;[174] seven tetratrico peptide repeats (TPRs; codons 6 to 279) that subserve rapsyn self-association;[174,175] a coiled-coil domain (codons 298 to 331) the hydrophobic surface of which can bind to determinants within the long cytoplasmic loop of each AChR subunit;[176] a Cys-rich RING-H2 domain (codons 363-402) that binds to the cytoplasmic domains of β-dystroglycan [177]and mediates the MuSK induced phosphorylation of AChR;[178]and a serine phosphorylation site at codon 406 (Fig. 8-25). Transcription of rapsyn in muscle is under the control of helix-loop-helix myogenic determination factors that bind to the *cis*-acting E-box sequence in the *RAPSN* promoter.[179]

CLINICAL FEATURES

In most patient, myasthenic symptoms present at birth or infancy; in a few it presents in the second or third decade.[180] Arthrogryposis at birth and other the congenital malformations occurs in nearly a third of the patients[23,180,181] but are not associated with specific mutations (Fig. 8-26).

Motor milestones are typically delayed and fatigable weakness persists during life. Respiratory infections or other intercurrent febrile illnesses precipitate increased weakness and respiratory crises and can result in anoxic encephalopathy.[23,180,182,183] Mutations in the open reading frame of *RAPSN* result in clinical features that resemble those of autoimmune myasthenia except for involvement of the extraocular muscles. Most patients have ptosis of varying severity that can be asymmetric, which is uncommon in other types of CMS.[184] Ophthalmoparesis is thought to be uncommon[180] but 9 of 39 patients in our series have had constant or episodic ophthalmoparesis.[185] Therefore absence of ophthalmoparesis is not a reliable criterion for distinguishing rapsyn-CMS from CMS caused by mutations in the AChR subunits or from autoimmune myasthenia gravis. Facial and bulbar weakness are common, often associated with neck muscle weakness. Proximal muscle weakness is more severe than distal weakness. Out-of-proportion weakness of the foot dorsiflexors was reported a feature of the late-onset phenotype[180] and was not detected in our series of early onset patients.[185]

Near-Eastern Jewish patients who carry an E-box mutation (-38A>G) in *RAPSN* have facial deformities associated with prognathism and malocclusion. They have mild to severe weakness of the masticatory muscles, moderate to severe eyelid ptosis without ophthalmoparesis, facial weakness, and slurred or hypernasal speech. Cervical, trunkal and limb muscles are usually spared.[179]

ELECTROPHYSIOLOGY

A decremental EMG response is present in some but not all patients. The decremental response on 2-Hz stimulation can appear only after subtetanic stimulation for 5 min or SFEMG is required to uncover the defect of neuromuscular transmission.[23] Similar EMG findings were reported in the Near-Eastern Jewish patients with facial malformations.[186,187]

In vitro electrophysiologic studies show a higher than normal quantal release in some patients. Consistent with the endplate AChR deficiency, the MEPP and MEPC amplitudes are reduced. Single-channel patch-clamp recordings show no kinetic abnormality of the AChR channel.[23]

MORPHOLOGY

The morphologic alterations resemble those in patients with low-expressor mutation of the AChR. At the light microscopic level, multiple small synaptic contacts are dispersed over an extended length of the muscle fiber (Fig. 8-27A). Immunostains of the EPs show reduced expression of rapsyn and a proportionately of AChR. The decrease in the number of AChRs per endplate is less marked than in patients with low-expressor mutation of the AChR.[23,185] Ultrastuctural studies show patchy expression of AChR on the shallow postsynaptic folds, few secondary clefts, and smaller than normal nerve terminals and postsynaptic regions, but the structural integrity of the pre- and postsynaptic regions is preserved[23,179] (Fig. 8-27B and C).

MOLECULAR FEATURES

Mutations have now been detected in the entre open reading frame and promoter region of *RAPSN*[23,179,180,188-192] (Fig. 8-25). Importantly, however, nearly all Indo-Europeans harbor a common N88K mutation.[190] Expression studies in different cell lines reveal that different rapsyn mutations hinder rapsyn colocalization with AChR, prevent formation of agrin-induced AChR clusters, impede rapsyn self-association, or reduce rapsyn expression.[191] Despite these differences, there are no consistent genotype-phenotype correlations except for that associated with homozygous -38A>G mutations.[185] For example, among two patients homozygous for the same N88K mutation, one had severe myasthenic symptoms and joint contractures at age 6 years

but the other had only mild weakness at age 27 years (Fig. 8-26). One patient heterozygous for N88K and L14P was as severely affected as another patient homozygous for N88K; and one patient who harbors N88K and 553ins5 and was born with arthrogryposis, but has only mild weakness at age 11.That identical mutations can have different phenotypic effects in different patients is likely due to polymorphisms in functionally related genes that can mitigate or worsen the effects of the mutations.

DIAGNOSIS

The diagnosis can be suspected on clinical grounds in presence congenital joint contractures or other malformations, worsening of symptoms and respiratory crises precipitated by febrile illness, and mild or no limitation of the ocular ductions. The definitive diagnosis depends on mutation analysis of *RAPSN*. In Indo-European patients this begins by screening for the N88K mutation but few Indo-Europeans do not carry this mutation; therefore, if the clinical history warrants, entire gene needs to be sequenced.[193,194] If this also fails to reveal a mutation, one needs to search for mutations in the long cytoplasmic loop of the δ subunit the consequences of which can mimic those of mutations in rapsyn.[164]

THERAPY

Most patients respond well to anticholinesterase medications; some derive additional benefit from the use of 3,4-DAP.[182,185] Some patients observed by the author benefited form the added use of ephedrine[182] or albuterol.

DEFECTS IN MECHANISMS GOVERNING ENDPLATE DEVELOPMENT AND MAINTENANCE

Since 2006, novel signaling and adapter molecules other than agrin and MuSK and novel pathways governing the development and maintenance of the EP have been identified. The newly identified molecules include Dok-7 (docking protein -7),[171] LRP4 (low-density lipoprotein receptor-related protein 4),[172,195] Crk and Crk-L (v-crk avian sarcoma virus CT10 oncogene homolog, and like Crk),[196] and Tid1 (a mammalian homolog of Drosophila tumorous imaginal discs).[197]

According to current understanding, Lrp4 is a coreceptor for agrin that mediates activation of MuSK by agrin.[172,195] MuSK activity is also regulated by the muscle-intrinsic protein Dok-7.[171] Tid1 is required for Dok7 to bind to MuSK.[197] Once recruited to MuSK, Dok-7 is phosphorylated by MuSK and activates MuSK via dimerization enhancing MuSK phosphorylation and MuSK kinase activity.[198] Agrin signaling also causes phosphorylation of two tyrosine residues in the C-terminal region of Dok-7; this leads to recruitment of the adapter proteins Crk and Crk-L that serve as downstream activators of Dok-7.[196,199] Each of the above proteins is a potential CMS target. Defects in MuSK, agrin, and Dok-7 are now known to cause CMS.

CMS caused by Defect in Agrin***

A homozygous G1709R mutation was identified in a 42-year-old woman with right lid ptosis since birth, no oculoparesis, and mild weakness of facial, hip-girdle and anterior tibial muscles, and refractoriness to pyridostigmine or 3,4-DAP.[200] The mutation is in the laminin G-like 2 domain, upstream of the *y* and *z* inserts of neural agrin required for MuSK activation and neuromuscular junction formation. AChR and agrin expression at the EP were normal. Structural studies showed EPs with misshaped synaptic gutters partially filled by nerve endings and

formation of new EP regions. The postsynaptic regions were preserved. Expression studies in myotubes using a mini-agrin construct revealed the mutation did not affect MuSK activation of agrin or agrin binding to α-dystroglycan. Forced expression of the mutant mini-agrin gene in mouse soleus muscle showed changes similar to those at patient EPs. Thus, the observed mutation perturbs the maintenance of the EP without altering the canonical function of agrin to induce development of the postsynaptic compartment.[200]

CMS Caused by Defects in MuSK

MuSK (a muscle specific receptor tyrosine kinase) under the influence of agrin, LRP4, Dok-7, Crk/Crk-L, and Tid1 regulates the development and maintenance of the EP and acts on rapsyn to concentrate AChR in the postsynaptic membrane.

Three reports document CMS caused by mutations in MuSK. The first report describes heteroallelic frameshift (220insC) and a missense (V790M) mutations in a patient with respiratory distress in early life, mild ptosis, decreased upward gaze, and fatigable weakness of the cervical and proximal more than distal muscles. The symptoms were worsened by pregnancy. Treatment with pyridostigmine and 3,4-DAP was ineffective.[201] The frameshift mutation prevents MuSK expression; the missense mutation decreases MuSK expression and impairs its interaction with Dok-7.[171] Forced expression of the mutant protein in mouse muscle decreased AChR expression at the EP and caused aberrant axonal outgrowth.[201] Interestingly, mice homozygous for MuSK V789M (which corresponds to the human MuSK V790M) are normal but mice hemizygous for V789M are severely affected; this suggests that MuSK V790M in humans is haploinsufficient only when accompanied by a null mutation.[202]

A second report describes heteroallelic M605I and A727V mutations in MuSK in a patient with severe myasthenic symptoms since early life that improved after puberty but worsened after menstrual periods. The MEPP and MEPC amplitudes in anconeus muscle were reduced to about 30% of normal and the EPP quantal content was half-normal. Synaptic contacts were small and electron microscopy showed simplified postsynaptic regions with too few secondary synaptic clefts. The patient failed to respond to pyridostigmine, ephedrine or 3,4-DAP but responded partially to albuterol.[203]

A third report describes a homozygous P31L mutation in the extracellular domain of MuSK in 5 patients in a consanguineous Sudanese kinship. The findings included ptosis from an early age, partial ophthalmoparesis, and weakness of torso and limb girdle muscles. Pyridostigmine therapy gave only slight benefit.[204]

CMS Caused by Defects in Dok-7

After the discovery in 2006 of Dok-7 as a muscle-intrinsic activator of MuSK,[171] numerous CMS-related mutations were identified in *DOK7* (see section below on Molecular Studies) and Dok-7 myasthenia is now recognized as a common cause of CMS.

Dok-7 is strongly expressed at the postsynaptic region of skeletal muscle and in heart. It harbors an N-terminal pleckstrin homology domain (PH) important for membrane association, a phosphotyrosine-binding (PTB) domain, and C-terminal sites for phosphorylation (Fig. 8-28A). The PTB and PH domains are required for association with and phosphorylation of MuSK. Phosphorylation of two of the C terminal residues is a requisite for Dok-7 activation by Crk and Crk-L.[196]

CLINICAL FEATURES AND RESPONSE TO THERAPY

The weakness in Dok-7 myasthenia typically has limb-girdle distribution but mild ptosis and facial weakness are not infrequent[15,205-210] (Fig. 8-29). Severe bulbar symptoms are uncommon